^{32}P primer for 14 V ent
Human spleen DNA

ng N. — ^{32}P 2633 (into the anchor primer)
follow P. 53 except use more ^{32}P ATP

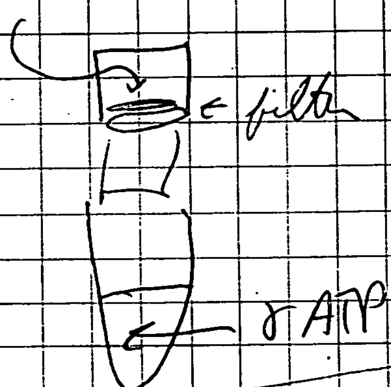
~26% primers have ATP is 100% efficiency in labeling

		①		②	
oligo 2633	159 µM	1 µl	✓ ✓	✓ (159 pm primer)	dry down
^{32}P γ ATP	6000 Ci/mmol	25 µl	✓ ✓	✓	1 IC6 ladder
10 mCi/µl 10-21-94 (1.67 µM ATP)				(41.8 pm ATP)	10 µl H_2O
					1 µl ^{32}P d CTP
5X Kinase buffer		6.75	✓ ✓	✓	15' 37°C
PNK 50 u/µl		0.25 µl	✓		1 µl EDTA
		33.75			

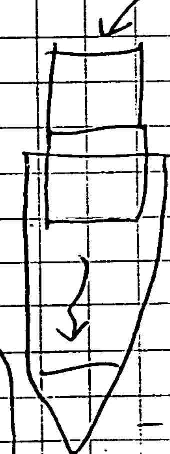
37°C 30 min → 5' 55°C → add

~~spin col same as P. 54, 7, and 145, 3~~

dilute ^{32}P 2633 with 100 µl H_2O (V_f = 133 now)
spin in microfuge in "micron 3"
(amicon # 42403) - after all went in, put
add 200 µl more H_2O and spin again
remove volume that did not enter filter



invert filter →



10-24-94

Had a problem - filter kept peeling
back on micron 3. Maybe g force was
too high on Beckman microfuge "E" model
will skip separation of free ATP.

^{32}P 2633 is diluted only 33.75 fold for
C_f = 4.71 µM

To Pag N .____

Witnessed & Understood by me,	Date	Invented by	Date
[signature]	10/24/94	[signature]	10-19-94
		Recorded by	10/24/94

[redacted]